

particularly sodium channel proteins, and voltage-gated sodium channel proteins.

The novel human nucleic acid sequences described herein, encode proteins/open reading frames (ORFs) of 1998, 1962, 1442, 1381, 1387, 2009, 1973, 1453, 1392, and 1398 amino acids in length (see respectively, SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20).

The invention also encompasses agonists and antagonists of the described NHPs, including small molecules, large molecules, mutant NHPs, or portions thereof that compete with native NHP, NHP peptides, and antibodies to the NHP, as well as nucleotide sequences that can be used to inhibit the expression of the described NHP (e.g., antisense and ribozyme molecules, and gene or regulatory sequence replacement constructs) or to enhance the expression of the described NHP ORF (e.g., expression constructs that place the described gene under the control of a strong promoter system), and transgenic animals that express a NHP transgene, or "knock-outs" (which can be conditional) that do not express a functional NHP.

Further, the present invention also relates to processes for identifying compounds that modulate, i.e., act as agonists or antagonists, of NHP expression and/or NHP activity that utilize purified preparations of the described NHPs and/or NHP product, or cells expressing the same. Such compounds can be used as therapeutic agents for the treatment of any of a wide variety of symptoms associated with biological disorders or imbalances.

4. DESCRIPTION OF THE SEQUENCE LISTING AND FIGURES

The Sequence Listing provides the sequences of the NHP ORFs encoding the described NHP amino acid sequences.

5. DETAILED DESCRIPTION OF THE INVENTION

The NHPs, described for the first time herein, are novel proteins that can be found expressed in, *inter alia*, human cell lines, human fetal brain, brain, pituitary,

cerebellum, spinal cord, thymus, spleen, lymph node, bone marrow, trachea, lung, kidney, fetal liver, liver, prostate, testis, thyroid, pancreas, salivary gland, stomach, small intestine, colon, skeletal muscle, heart,
5 uterus, placenta, mammary gland, adrenal gland, hypothalamus, adipose, esophagus, bladder, cervix, pericardium, ovary, fetal kidney, fetal lung, and mammalian gene trapped cells.

The present invention encompasses the nucleotides
10 presented in the Sequence Listing, host cells expressing such nucleotides, the expression products of such nucleotides, and: (a) nucleotides that encode mammalian homologs of the described genes, including the specifically described NHP, and the NHP products; (b) nucleotides that
15 encode one or more portions of the NHP that correspond to functional domains, and the polypeptide products specified by such nucleotide sequences, including but not limited to the novel regions of any active domain(s); (c) isolated nucleotides that encode mutant versions, engineered or
20 naturally occurring, of a NHP in which all or a part of at least one domain is deleted or altered, and the polypeptide products specified by such nucleotide sequences, including but not limited to soluble proteins and peptides in which all or a portion of the signal sequence is deleted; (d)
25 nucleotides that encode chimeric fusion proteins containing all or a portion of a coding region of NHP, or one of its domains (e.g., a receptor or ligand binding domain, accessory protein/self-association domain, etc.) fused to another peptide or polypeptide; or (e) therapeutic or
30 diagnostic derivatives of the described polynucleotides such as oligonucleotides, antisense polynucleotides, ribozymes, dsRNA, or gene therapy constructs comprising a sequence first disclosed in the Sequence Listing.

As discussed above, the present invention includes:
35 (a) the human DNA sequences presented in the Sequence Listing (and vectors comprising the same) and additionally contemplates any nucleotide sequence encoding a contiguous

sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. *et al.*, eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a functionally equivalent gene product. Additionally contemplated are any nucleotide sequences that hybridize to the complement of a DNA sequence that encodes and expresses an amino acid sequence presented in the Sequence Listing under moderately stringent conditions, *e.g.*, washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel *et al.*, 1989, *supra*), yet still encodes a functionally equivalent NHP product. Functional equivalents of a NHP include naturally occurring NHPs present in other species and mutant NHPs whether naturally occurring or engineered (by site directed mutagenesis, gene shuffling, directed evolution as described in, for example, U.S. Patent No. 5,837,458). The invention also includes degenerate nucleic acid variants of the disclosed NHP polynucleotide sequences.

Additionally contemplated are polynucleotides encoding a NHP ORF, or its functional equivalent, encoded by polynucleotide sequences that are about 99, 95, 90, or about 85 percent similar or identical to corresponding regions of the nucleotide sequences of the Sequence Listing (as measured by BLAST sequence comparison analysis using, for example, the GCG sequence analysis package using standard default settings).

The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the described NHP gene (or coding region) nucleotide sequences. Such hybridization conditions may be highly stringent or less highly stringent, as described above. In instances where the

Alternatively, such NHP oligonucleotides can be used as hybridization probes for screening libraries, and assessing gene expression patterns (particularly using a micro array or high-throughput "chip" format).

Additionally, a series of the described NHP oligonucleotide sequences, or the complements thereof, can be used to represent all or a portion of the described NHP sequences. The oligonucleotides, typically between about 16 to about 40 (or any whole number within the stated range) nucleotides in length can partially overlap each other and/or the NHP sequence may be represented using oligonucleotides that do not overlap. Accordingly, the described NHP polynucleotide sequences shall typically comprise at least about two or three distinct oligonucleotide sequences of at least about 18, and preferably about 25, nucleotides in length that are each first disclosed in the described Sequence Listing. Such oligonucleotide sequences may begin at any nucleotide present within a sequence in the Sequence Listing and proceed in either a sense (5'-to-3') orientation vis-a-vis the described sequence or in an antisense orientation.

For oligonucleotide probes, highly stringent conditions may refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). These nucleic acid molecules may encode or act as NHP gene antisense molecules, useful, for

example, in NHP gene regulation (for and/or as antisense primers in amplification reactions of NHP gene nucleic acid sequences). With respect to NHP gene regulation, such techniques can be used to regulate biological functions.

- 5 Further, such sequences may be used as part of ribozyme and/or triple helix sequences that are also useful for NHP gene regulation.

Inhibitory antisense or double stranded oligonucleotides can additionally comprise at least one
 10 modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine,
 15 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine,
 20 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine,
 25 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

- 30 The antisense oligonucleotide can also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense
 35 oligonucleotide will comprise at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a

phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier *et al.*, 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue *et al.*, 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue *et al.*, 1987, FEBS Lett. 215:327-330). Alternatively, double stranded RNA can be used to disrupt the expression and function of a targeted NHP. Oligonucleotides of the invention can be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein *et al.* (1988, Nucl. Acids Res. 16:3209), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*, 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

Low stringency conditions are well known to those of skill in the art, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual (and periodic updates thereof), Cold Springs Harbor Press, N.Y.; and Ausubel *et al.*, 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y.

Alternatively, suitably labeled NHP nucleotide probes can be used to screen a human genomic library using appropriately stringent conditions or by PCR. The

identification and characterization of human genomic clones is helpful for identifying polymorphisms (including, but not limited to, nucleotide repeats, microsatellite alleles, single nucleotide polymorphisms, or coding single
 5 nucleotide polymorphisms), determining the genomic structure of a given locus/allele, and designing diagnostic tests. For example, sequences derived from regions adjacent to the intron/exon boundaries of the human gene can be used to design primers for use in amplification
 10 assays to detect mutations within the exons, introns, splice sites (e.g., splice acceptor and/or donor sites), etc., that can be used in diagnostics and pharmacogenomics.

Further, a NHP gene homolog can be isolated from nucleic acid from an organism of interest by performing PCR
 15 using two degenerate or "wobble" oligonucleotide primer pools designed on the basis of amino acid sequences within the NHP products disclosed herein. The template for the reaction may be total RNA, mRNA, and/or cDNA obtained by reverse transcription of mRNA prepared from human or non-
 20 human cell lines or tissue known or suspected to express an allele of a NHP gene. The PCR product can be subcloned and sequenced to ensure that the amplified sequences represent the sequence of the desired NHP gene. The PCR fragment can then be used to isolate a full length cDNA clone by a
 25 variety of methods. For example, the amplified fragment can be labeled and used to screen a cDNA library, such as a bacteriophage cDNA library. Alternatively, the labeled fragment can be used to isolate genomic clones via the screening of a genomic library.

30 PCR technology can also be used to isolate full length cDNA sequences. For example, RNA can be isolated, following standard procedures, from an appropriate cellular or tissue source (i.e., one known, or suspected, to express a NHP gene). A reverse transcription (RT) reaction can be
 35 performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting

RNA/DNA hybrid may then be "tailed" using a standard terminal transferase reaction, the hybrid may be digested with RNase H, and second strand synthesis may then be primed with a complementary primer. Thus, cDNA sequences
5 upstream of the amplified fragment can be isolated. For a review of cloning strategies that can be used, see e.g., Sambrook *et al.*, 1989, *supra*.

A cDNA encoding a mutant NHP gene can be isolated, for example, by using PCR. In this case, the first cDNA strand
10 may be synthesized by hybridizing an oligo-dT oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an individual putatively carrying a mutant NHP allele, and by extending the new strand with reverse transcriptase. The second strand of
15 the cDNA is then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the normal gene. Using these two primers, the product is then amplified via PCR, optionally cloned into a suitable vector, and subjected to DNA sequence analysis through methods well
20 known to those of skill in the art. By comparing the DNA sequence of the mutant NHP allele to that of a corresponding normal NHP allele, the mutation(s) responsible for the loss or alteration of function of the mutant NHP gene product can be ascertained.

25 Alternatively, a genomic library can be constructed using DNA obtained from an individual suspected of or known to carry a mutant NHP allele (e.g., a person manifesting a NHP-associated phenotype such as, for example, obesity, high blood pressure, connective tissue disorders,
30 infertility, diabetes, alopecia, arrhythmia, etc.), or a cDNA library can be constructed using RNA from a tissue known, or suspected, to express a mutant NHP allele. A normal NHP gene, or any suitable fragment thereof, can then be labeled and used as a probe to identify the
35 corresponding mutant NHP allele in such libraries. Clones containing mutant NHP gene sequences can then be purified

and subjected to sequence analysis according to methods well known to those skilled in the art.

Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from a tissue known, or suspected, to express a mutant NHP allele in an individual suspected of or known to carry such a mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against a normal NHP product, as described below. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.)

Additionally, screening can be accomplished by screening with labeled NHP fusion proteins, such as, for example, AP-NHP or NHP-AP fusion proteins. In cases where a NHP mutation results in an expressed gene product with altered function (e.g., as a result of a missense or a frameshift mutation), polyclonal antibodies to a NHP are likely to cross-react with a corresponding mutant NHP gene product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis according to methods well known in the art.

The invention also encompasses (a) DNA vectors that contain any of the foregoing NHP coding sequences and/or their complements (*i.e.*, antisense); (b) DNA expression vectors that contain any of the foregoing NHP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences (for example, baculo virus as described in U.S. Patent No. 5,869,336 herein incorporated by reference);

(c) genetically engineered host cells that contain any of the foregoing NHP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell; and (d) genetically engineered host cells that express an endogenous NHP gene

under the control of an exogenously introduced regulatory element (i.e., gene activation). As used herein, regulatory elements include but are not limited to inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, regulatable, viral (particularly retroviral LTR promoters) the early or late promoters of SV40 adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *tet* system, the *TRC* system, the major operator and promoter regions of phage lambda, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase (PGK), the promoters of acid phosphatase, and the promoters of the yeast α -mating factors.

The present invention also encompasses antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists and agonists of the NHP, as well as compounds or nucleotide constructs that inhibit expression of a NHP gene (transcription factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence replacement constructs), or promote the expression of a NHP (e.g., expression constructs in which NHP coding sequences are operatively associated with expression control elements such as promoters, promoter/enhancers, etc.).

The NHP or NHP peptides, NHP fusion proteins, NHP nucleotide sequences, antibodies, antagonists and agonists can be useful for the detection of mutant NHPs or inappropriately expressed NHPs for the diagnosis of disease. The NHP proteins or peptides, NHP fusion proteins, NHP nucleotide sequences, host cell expression systems, antibodies, antagonists, agonists and genetically engineered cells and animals can be used for screening for drugs (or high throughput screening of combinatorial libraries) effective in the treatment of the symptomatic or phenotypic manifestations of perturbing the normal function of NHP in the body. The use of engineered host cells

5.1 THE NHP SEQUENCES

The cDNA sequences and the corresponding deduced amino acid sequences of the described NHPs are presented in the Sequence Listing. The NHP nucleotides were obtained from
5 cDNAs generated from human brain, fetal brain, pituitary, cerebellum, testis, prostate, fetal brain, adipose mRNAs (Clontech, Palo Alto, CA, Edge Biosystems, Gaithersburg, MD).

Several polymorphisms were identified including an A-
10 or-C transversion in the sequence region corresponding to, for example, nucleotide number 2,941 of SEQ ID NO:1 which can result in either a leu or met being present in the corresponding amino acid sequence region represented by, for example, amino acid position number 981 of SEQ ID NO:2,
15 and a A-or-G transition in the sequence region corresponding to, for example, nucleotide number 3,166 of SEQ ID NO:1 which can result in either a thr or ala being present in the corresponding amino acid sequence region represented by, for example, amino acid position number
20 1,056 of SEQ ID NO:2.

5.2 THE NHP AND NHP POLYPEPTIDES

The described NHPs, NHP polypeptides, peptide fragments, mutated, truncated, or deleted forms of the
25 NHPs, and/or NHP fusion proteins can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, the identification of other cellular gene products related to NHP, as reagents in assays for screening for compounds
30 that can be used as pharmaceutical reagents useful in the therapeutic treatment of mental, biological, or medical disorders and diseases. Given the similarity information and expression data, the described NHP can be targeted (by drugs, oligos, antibodies, etc,) in order to treat disease,
35 or to therapeutically augment the efficacy of, for example, chemotherapeutic agents.

The Sequence Listing discloses the amino acid sequence encoded by the described NHP ORF. The NHPs display initiator methionines in DNA sequence contexts consistent with translation initiation sites.

5 The NHP amino acid sequences of the invention includes the amino acid sequence presented in the Sequence Listing as well as analogues and derivatives thereof. Further, corresponding NHP homologues from other species are encompassed by the invention. In fact, any NHP proteins
10 encoded by the NHP nucleotide sequences described above are within the scope of the invention, as are any novel polynucleotide sequences encoding all or any novel portion of an amino acid sequence presented in the Sequence Listing. The degenerate nature of the genetic code is well
15 known, and, accordingly, each amino acid presented in the Sequence Listing, is generically representative of the well known nucleic acid "triplet" codon, or in many cases codons, that can encode the amino acid. As such, as contemplated herein, the amino acid sequences presented in
20 the Sequence Listing, when taken together with the genetic code (see, for example, Table 4-1 at page 109 of "Molecular Cell Biology", 1986, J. Darnell et al. eds., Scientific American Books, New York, NY, herein incorporated by reference) are generically representative of all the
25 various permutations and combinations of nucleic acid sequences that can encode such amino acid sequences.

The invention also encompasses proteins that are functionally equivalent to the NHP encoded by the presently described nucleotide sequences as judged by any of a number
30 of criteria, including, but not limited to, the ability to bind and cleave a substrate of a NHP, or the ability to effect an identical or complementary downstream pathway, or a change in cellular metabolism (e.g., proteolytic activity, ion flux, tyrosine phosphorylation, etc.). Such
35 functionally equivalent NHP products include, but are not limited to, additions or substitutions of amino acid residues within the amino acid sequence encoded by the NHP

nucleotide sequences described above, but which result in a silent change, thus producing a functionally equivalent gene product. Amino acid substitutions can be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

A variety of host-expression vector systems can be used to express the NHP nucleotide sequences of the invention. Where, as in the present instance, the NHP peptide or polypeptide is thought to be membrane protein, the hydrophobic regions of the protein can be excised and the resulting soluble peptide or polypeptide can be recovered from the culture media. Such expression systems also encompass engineered host cells that express a NHP, or functional equivalent, *in situ*. Purification or enrichment of a NHP from such expression systems can be accomplished using appropriate detergents and lipid micelles and methods well known to those skilled in the art. However, such engineered host cells themselves may be used in situations where it is important not only to retain the structural and functional characteristics of the NHP, but to assess biological activity, e.g., in drug screening assays.

The expression systems that may be used for purposes of the invention include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing NHP nucleotide sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors

containing NHP nucleotide sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing NHP sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing NHP nucleotide sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the NHP product being expressed. For example, when a large quantity of such a protein is to be produced for the generation of pharmaceutical compositions of or containing NHP, or for raising antibodies to a NHP, vectors that direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, EMBO J. 2:1791), in which a NHP coding sequence may be ligated individually into the vector in frame with the *lacZ* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Pharmacia or American Type Culture Collection) can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to include thrombin or factor Xa protease cleavage

In an insect system, *Autographa californica* nuclear polyhydrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. A NHP gene coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

Successful insertion of NHP gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed (*e.g.*, see Smith *et al.*, 1983, *J. Virol.* 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the NHP nucleotide sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a NHP product in infected hosts (*e.g.*, See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted NHP nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire NHP gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a

portion of a NHP coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (See Bitter *et al.*, 1987, *Methods in Enzymol.* 153:516-544).

In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, human cell lines.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the NHP sequences described above can be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer sequences, transcription terminators, polyadenylation